INVITED REVIEW

Plant Allometry, Leaf Nitrogen and Phosphorus Stoichiometry, and Interspecific Trends in Annual Growth Rates

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● *Background* Life forms as diverse as unicellular algae, zooplankton, vascular plants, and mammals appear to obey quarter-power scaling rules. Among the most famous of these rules is Kleiber's (i.e. basal metabolic rates scale as the three-quarters power of body mass), which has a botanical analogue (i.e. annual plant growth rates scale as the three-quarters power of total body mass). Numerous theories have tried to explain why these rules exist, but each has been heavily criticized either on conceptual or empirical grounds.
● *N,P-Stoichiometry* Recent models predicting growth rates on the basis of how total cell, tissue, or organism nitrogen and phosphorus are allocated, respectively, to protein and rRNA contents may provide the answer, particularly in light of the observation that annual plant growth rates scale linearly with respect to standing leaf mass and that total leaf mass scales isometrically with respect to nitrogen but as the three-quarters power of leaf phosphorus. For example, when these relationships are juxtaposed with other allometric trends, a simple N,P-stoichiometric model successfully predicts the relative growth rates of 131 diverse C_3 and C_4 species.
● *Conclusions* The melding of allometric and N,P-stoichiometric theoretical insights provides a robust modelling approach that conceptually links the subcellular 'machinery' of protein/ribosomal metabolism to observed growth rates of uni- and multicellular organisms. Because the operation of this 'machinery' is basic to the biology of all life forms, its allometry may provide a mechanistic explanation for the apparent ubiquity of quarter-power scaling rules.

Key words: Biomass allocation, Dobberfuhl models, leaf chemistry, leaf protein investment, relative growth rates, quarter-power scaling rules, ribosomal RNA.

INTRODUCTION

Numerous quarter-power scaling rules appear to span all levels of biological organization, from molecules to eco-systems, across pro- and eukaryotes, plants and animals (Hemmingsen, 1960; Peters, 1983; Calder, 1984, 1996; Schmidt-Nielsen, 1984). For example, across animal species ranging in size from that of a mouse to an elephant, maximum life span in captivity, blood volume circulation, fast muscle contraction, and a host of other phenomena, each scale closely to the one-quarter power of body mass (Lindstedt and Calder, 1981). Perhaps the most famous of these rules is Kleiber's, which states that basal metabolic rates scale as the three-quarter power of body mass (Kleiber, 1932, 1961)—a scaling relationship that finds its analogue in the allometry of growth rate versus body mass across the polyphyletically and ecologically diverse unicellular algae and terrestrial vascular embryophytes (Banse, 1976; Niklas, 1994; Niklas and Enquist, 2001).

Yet, the identification of an unambiguous mechanistic explanation for the origin of these scaling rules remains an open theoretical problem. Numerous explanations have been advanced, but each has been viewed with a critical if not jaundiced eye (e.g. Blaxter, 1965; Blum, 1977; Gray, 1981; Economos, 1982, 1983; Heusner, 1982; Feldman and McMahon, 1983; Feldman, 1995; Prothero, 1986*a*). Among the most recent of these theories is that of West, Brown and Enquist, who assert that all quarter-power scaling rules (and their one-quarter multiples such as three-quarters) emerge

from the interplay between the physical or geometric con-straints resulting from three functional properties of every biological network transport system (West *et al.*, 1997, 1999, 2001). Specifically, their theory (the 'WBE' theory) claims that all networks (*a*) are space-filling, hierarchical branching systems, (*b*) have terminal branch elements that are invariant in size, and (*c*), by virtue of natural selection, minimize the energy required to transport and deliver nutri-ents (and thus minimize either the time or distance nutrients are moved).

As so many theories before it, the WBE theory has been heavily criticized on empirical, theoretical and even strictly mathematical grounds (e.g. Dodds *et al.*, 2001; Darveau *et al.*, 2002; Weibel, 2002). Arguably, the first assumption (i.e. that biological delivery networks are 'fractal' in nature) is consistent with the 'self-similarity' typically observed when branched nutrient networks within multicellular organisms are dissected and numerically quantified. How-ever, if the WBE theory is valid across all levels of bio-logical organization, from that of molecules to ecosystems as claimed by its authors, fractal-like delivery networks must exist at each level. This is difficult to imagine for some (e.g. molecules) and undocumented for others (e.g. organelles and ecosystems). Similar concerns exist for the two remain-ing assumptions of the WBE theory, e.g. it has yet to be established that capillaries, bronchioles and terminal xylary elements are invariant in size or that they minimize the time and energy required to exchange mass or energy.

Despite these concerns (or perhaps because of them), the WBE theory has engendered a renaissance in the field of

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allometric theory and empirical enquiry—one in which alternative theories for the existence of quarter-power scaling rules continue to be sought. It is in this context that recent developments in modelling the effects of nitrogen and phosphorus allocation patterns on protein synthesis rates and thus 'growth' are particularly exciting. These models emerge from this perspective that, irrespective of phyletic affinity or ecological preference, the growth rate of any kind of organism is positively correlated with ribosome number and rate of activity and negatively correlated with protein concentration (Dobberfuhl, 1999; Sterner and Elser, 2002; Elser *et al.*, 2003; Ågren, 2004; Vrede *et al.*, 2004). Conceptually, the amounts of ribosomes and proteins are thought of as respective measures of an organism's protein-production 'machinery' and the 'overhead' that must be produced per unit time to maintain a constant growth rate. Nitrogen and phosphorus (N and P) stoichiometry is emphasized, because large fractions of the total N and P are allocated to the construction of proteins and rRNA. Thus, N,P-stoichiometry is predicted to correlate with growth rate at the level of cells, tissues or the whole organism (Dobberfuhl, 1999; Sterner and Elser, 2002; Vrede *et al.*, 2004). Specifically, growth rates should correlate positively with increasing rRNA (and P) investments relative to protein (and N) investments.

This prediction is particularly relevant to three previously reported allometric relationships for plants (Niklas and Enquist, 2001, 2002). First, annual growth rates in body mass across phyletically and ecologically diverse species appear to scale as the three-quarters power of body size. Secondly, growth rates scale linearly (isometrically) with the capacity to intercept sunlight. And, thirdly, total leaf N appears to scale as the three-quarters power of total leaf P, across and within some species (Niklas and Cobb, 2005; Niklas *et al.*, 2005). The goal of this paper is to review these relationships and to explore them empirically with the aid of a recently expanded database for non-woody and woody plant species ranging across 11 orders of magnitude in total body size.

A STATISTICAL PROLEGOMENA

However, before proceeding with this review, it is important to consider first how allometric scaling relationships are adduced statistically, particularly because these techniques are used to evaluate the numerical parameters that describe all allometric relationships, including those discussed throughout this paper (e.g. Tables 1 and 2).

Each of the biological scaling relationships referred to as 'power rules' complies mathematically with the formula

$$Y_o = \beta Y_a^\alpha \qquad (1)$$

where Y_o and Y_a are the variables plotted on the ordinate and abscissa axes, respectively, β is the normalization constant and α is the scaling exponent. In most cases, but not all, Y_a is some measure of mass (typically but not invariably expressed in units of carbon mass). When $\alpha = 1$, eqn (1) describes an isometric relationship, i.e. one that plots as a straight line on both linear and logarithmic axes. When

TABLE 1. *Reduced major axis regression scaling exponents, allometric constants, and their respective 95 % confidence intervals (see eqns 3–6) for log$_{10}$-transformed annual growth rates* G$_T$, *light interception capabilities* H, *and total dry body mass* M$_T$ *of unicellular algae, non-woody plants, and woody plants (see Figs 1 and 2)*

	α_{RMA} (95 % CI)	log β_{RMA} (95 % CI)	r^2	F
Unicellular algae (*H* gauged by cell pigment concentration C_P), $N = 68$				
H vs. G_T	0·95 (0·87; 1·03)	−3·51 (−4·43; −2·60)	0·886	488
G_T *vs.* M_T	0·75 (0·73; 0·76)	−0·91 (−1·10; −0·70)	0·995	9745
H vs. M_T	0·71 (0·64; 0·78)	−4·38 (−5·25; −3·50)	0·876	453
Non-woody species (*H* gauged by standing leaf mass M_L), $N = 1147$				
H vs. G_T	1·01 (0·97; 1·06)	−0·91 (−1·01; −0·80)	0·903	3957
G_T *vs.* M_T	0·99 (0·95; 1·04)	0·51 (0·39; 0·63)	0·907	3046
H vs. M_T	1·01 (0·99; 1·03)	−0·39 (−0·46; −0·32)	0·975	8144
Woody species (*H* gauged by standing leaf mass M_L), $N = 265$				
H vs. G_T	0·90 (0·84; 0·97)	−0·11 (−0·19; −0·03)	0·790	545
G_T *vs.* M_T	0·77 (0·71; 0·83)	−0·74 (−0·87; −0·61)	0·804	581
H vs. M_T	0·70 (0·64; 0·75)	−0·78 (−0·91; −0·65)	0·766	1065

TABLE 2. *Reduced major axis regression scaling exponents, allometric constants, and their respective 95 % confidence intervals (see eqns 3–6) for log$_{10}$–transformed data of total leaf nitrogen, phosphorus, and carbon content (*M$_{LN}$, M$_{LP}$ *and* M$_{LC}$, *respectively*)*

	α_{RMA} (95 % CI)	log β_{RMA} (95 % CI)	r^2	F
Across herbaceous species, $N = 131$				
M_{LN} *vs.* M_{LC}	1·06 (0·95; 1·17)	−1·67 (−1·76; −1·58)	0·941	2058
M_{LP} *vs.* M_{LC}	1·37 (1·27; 1·48)	−2·61 (−2·70; −2·52)	0·968	3881
M_{LN} *vs.* M_{LP}	0·78 (0·72; 0·85)	−0·74 (−0·72; −0·76)	0·948	2339
Eranthis hyemalis, $N = 17$				
M_{LN} *vs.* M_{LC}	1·00 (0·98; 1·03)	−1·33 (−1·39; −1·26)	0·996	3929
M_{LP} *vs.* M_{LC}	1·37 (1·32; 1·42)	0·77 (0·65; 0·90)	0·993	2283
M_{LN} *vs.* M_{LP}	0·73 (0·70; 0·76)	−1·89 (−1·97; −1·82)	0·996	3425
Across Reich and Oleksyn (2004) data set, $N = 7445$				
M_{LN} *vs.* M_{LP}	0·73 (0·71; 0·75)	1·08 (1·07; 1·08)	0·335	3753

$\alpha \neq 1$, eqn (1) describes an allometric relationship, i.e. one that plots as a linear function on logarithmic axes. Logarithmic transformation of eqn (1) shows that logβ and α are the Y_o-intercept and the slope of the log–log linear allometric relationship, respectively, i.e.

$$\log Y_o = \log \beta + \alpha \log Y_a \qquad (2)$$

The linearization of data by means of logarithmic transformation has become a conventional practice in allometric studies, in part because it minimizes the sum of squared residuals for the transformed as opposed to the original function. It should be noted, however, that regression parameters estimated in this way do not invariably provide the best fit of data to a regression model compared with minimizing the squared residuals for the actual function by using nonlinear regression protocols. Analyses of residuals are required to determine whether log–log linear or log–log nonlinear functions optimize the goodness-of-fit. This protocol does not appear to be a 'standard practice', perhaps because most allometric theories assert (or require) the existence of numerically unique scaling exponents, which do not exist for log–log nonlinear relationships.

The objective of the vast majority of allometric studies is to determine the numerical values of $\log\beta$ and α. When a predictive relationship is sought, simple ordinary least squares (OLS) regression analysis can be used. When the objective is to establish a functional relationship between Y_o and Y_a, as is generally in case, OLS regression analysis is ill equipped for this purpose, in part because it is based on the assumption that Y_a is biologically independent of Y_o and that it is measured without error. Three regression methods have been suggested to overcome this limitation, i.e. Bartlett's three-group method, principal axis regression, and reduced major axis regression (Sokal and Rohlf, 1980). Considerable controversy revolves around which of these methods is the most appropriate (Smith, 1980; Harvey, 1982; Seim, 1983; Rayner, 1985; Prothero, 1986*b*; McArdle, 1988, 2003; Jolicoeur, 1990). This issue is not trivial, especially when the goal is to 'test' when empirically determined scaling exponents agree statistically with those predicted by a particular theory, because the numerical values of α and $\log\beta$ depend on the regression techniques used and because different techniques can produce significantly different numerical values even for the same data set.

Space precludes a detailed discussion of the merits and detractions of each of the three regression methods. However, reduced major axis (RMA) regression analysis has emerged as a 'standard' allometric technique over the past few years. Statistical software is available to perform RMA regression analyses, but access to this software is not critical, because OLS regression summary statistics provide all the necessary information to compute the numerical values of α and $\log\beta$, and their corresponding 95 % confidence intervals.

Specifically, these regression parameters can be computed using the formulas

$$\alpha_{RMA} = \alpha_{OLS}/r \tag{3}$$

and

$$\log\beta_{RMA} = \overline{\log Y_o} - \alpha_{RMA}\,\overline{\log Y_a} \tag{4}$$

where α_{RMA} is the (reduced major axis) scaling exponent, α_{OLS} is the OLS regression slope, r is the OLS correlation coefficient, $\log\beta_{RMA}$ is now called the allometric constant, and $\overline{\log Y}$ denotes the mean value of $\log Y$. The corresponding 95 % confidence intervals of these two regression parameters are computed using the formulas

$$\alpha_{RMA} \pm t_{N-2}\left(\frac{MSE}{SS_a}\right)^{1/2} \tag{5}$$

and

$$\log\beta_{RMA} \pm t_{N-2}\left[MSE\left(\frac{1}{N} + \frac{\overline{\log Y_a^2}}{SS_a}\right)\right]^{1/2} \tag{6}$$

where *MSE* is the OLS mean square error, SS_a is the OLS sums of squares for $\log Y_a$, N is the sample size, and $t_{N-2} = 1\cdot96$ when $N - 2 > 120$.

Eqns (3)–(6) are used throughout this paper to evaluate the numerical values of scaling exponents, allometric constants, and their respective 95 % confidence intervals (see Tables 1 and 2). As noted, the data base used to establish these numerical values has been recently expanded in terms of species number, body size range and phylogenetic diversity compared with that used previously to establish scaling exponents (e.g. Niklas, 1994, 2004; Niklas and Enquist, 2001, 2002).

LIGHT, GROWTH AND BODY SIZE

Two scaling relationships appear to cut across phyletically diverse unicellular algae and tree-sized embryophytes. Growth in dry mass per individual per year ('annual growth', G_T) scales isometrically with respect to the capacity to intercept sunlight (quantified by pigment concentration per cell for unicellular algae, C_P, and by standing leaf mass for tree species, M_L), and annual growth scales as the three-quarters power of body mass (total cell or organism dry mass, M_T) (Banse, 1976; Niklas, 1994, 2004; Niklas and Enquist, 2001). Respectively, these scaling relationships are expressed by the isometric and allometric formulas

$$H = \beta_0 G_T \tag{7}$$

and

$$G_T = \beta_1 M_T^{3/4} \tag{8}$$

where H denotes C_P or M_L and allometric constants are distinguished from each other by different numerical subscripts. Combining eqns (7) and (8) the prediction is obtained that the ability to harvest sunlight as gauged by C_P or M_L should remain proportional to the three-quarters power of total body mass, i.e.

$$H = \beta_2 M_T^{3/4} \tag{9}$$

where $\beta_2 = \beta_0\beta_1$. These log–log linear scaling relationships are illustrated in Figs 1 and 2 with newly acquired data.

The isometric relationship between H and G_T represented by eqn (7) makes some intuitive sense. Even though the ability to 'harvest sunlight' and its corresponding 'energy use efficiency' are very different biophysical phenomena, it is not unreasonable to expect growth rates to correlate linearly with the ability to capture radiant energy. In contrast, it is far less obvious why either annual growth rate or light-harvesting ability should scale as the three-quarters power of body mass. Early workers exploring the relationship between basal metabolic rates across animals differing in body size expected a two-thirds scaling exponent, because it was assumed that the ability of cells or entire organisms to exchange mass or energy with the environment is governed by body surface area (which scales as the square of any linear reference dimension L) and that the demand for nutrients is gauged by body volume (which scales as the cube of L). Of course the two-thirds scaling relationship between surface area and volume holds true only for a series of geometrically identical objects that retain the
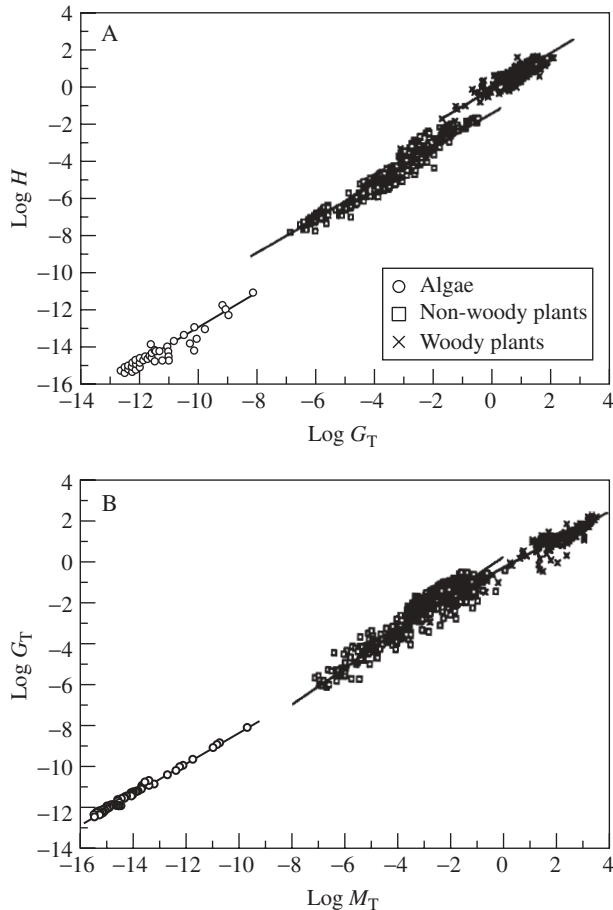
FIG. 1. Log–log bivariate plots illustrating the allometric relationships between the capacity to harvest sunlight H, annual growth rate G_T, and total body mass M_T for unicellular algae and non-woody and woody plants (for an explanation of symbols, see insert in A). Lines are reduced major axis regression curves (for a summary of regression statistics, see Table 1).
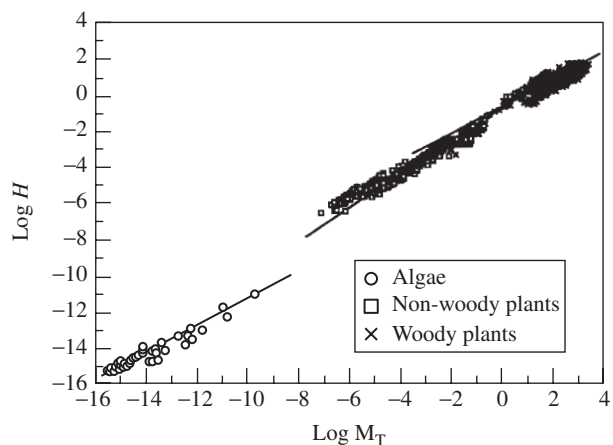

FIG. 2. Log–log bivariate plot illustrating the allometric relationship between the capacity to harvest sunlight H and total body mass M_T for unicellular algae and non-woody and woody plants (for an explanation of symbols, see insert). Lines are reduced major axis regression curves (for a summary of regression statistics, see Table 1).

same shape as they increase in size—two conditions that are repeatedly violated by unicellular and multicellular organisms, either ontogenetically or phylogenetically.

Regardless of the mechanistic explanation for why the three scaling relationships exist, each receives reasonably strong statistical support when 'tested' against empirically observed trends for phylogenetically diverse unicellular algae and tree-sized dicots and conifers (Table 1). For these organisms, the 95 % confidence intervals of the slope of the log–log linear relationship between light harvesting capability and annual growth approach or include unity. Likewise, the intervals of the slope of the log–log linear relationship between annual growth and total body mass include 0·75. Thus, the proportional relationships summarized by $H \propto G_T \propto M_T^{3/4}$ are reasonably accurate for unicellular algae and tree-sized plants.

In pointed contrast, the allometry of non-woody plants (i.e. herbaceous species and 1-year old-dicot and conifer tree species) deviates from that predicted by eqns (8) and (9) and observed for unicellular algae and tree-sized individuals, because it is strongly isometric in terms of all three biological variables, i.e. $H \propto G_T \propto M_T$ (Table 1). In this sense, the three-quarters scaling 'rule' asserted for all unicellular and multicellular plants and animals is not 'invariant' as is sometimes claimed (although it cannot escape attention that isometry can emerge as a 'quarter-power rule' in the most general sense).

LEAF N, P STOICHIOMETRY

That growth does not invariably scale as the three-quarters power of body mass is evident from the analyses of data for non-woody vascular plants presented in the previous section. Nevertheless, the claim that annual growth across ecologically and phyletically diverse unicellular and multicellular photoautotrophic eukaryotes scales isometrically or nearly so with respect to light-harvesting ability (see Niklas and Enquist, 2001, 2002) is statistically robust (Table 1). In the case of unicellular photoautotrophs, H is measured in units of photosynthetic pigment concentrations per cell, C_P. However, for terrestrial embryophytes, H is measured in terms of standing dry leaf mass per plant, M_L. Thus, annual growth appears to be inexorably linked to the 'machinery' of photosynthesis in some very basic way that cuts across otherwise sharply defined phyletic boundaries.

This linkage probably exists at numerous metabolic and structural levels, but the view advocated here is that it is sensitive to the manner in which nitrogen and phosphorus is allocated in light-harvesting structures. This perspective is based on the comparatively strong scaling relationships that exist between total leaf carbon mass (M_{LC}) and total leaf nitrogen and phosphorus (M_{LN} and M_{LP}, respectively)—relationships that appear to obey their own quarter-power 'rules' across and within those species that have been examined in sufficient detail.

For example, based on stoichiometric data collected from 131 herbaceous species, including C_3 and C_4 species, Niklas *et al.* (2005) report that leaf nitrogen content scales almost isometrically with respect to increasing leaf carbon content, whereas leaf phosphorus content scales as the four-thirds
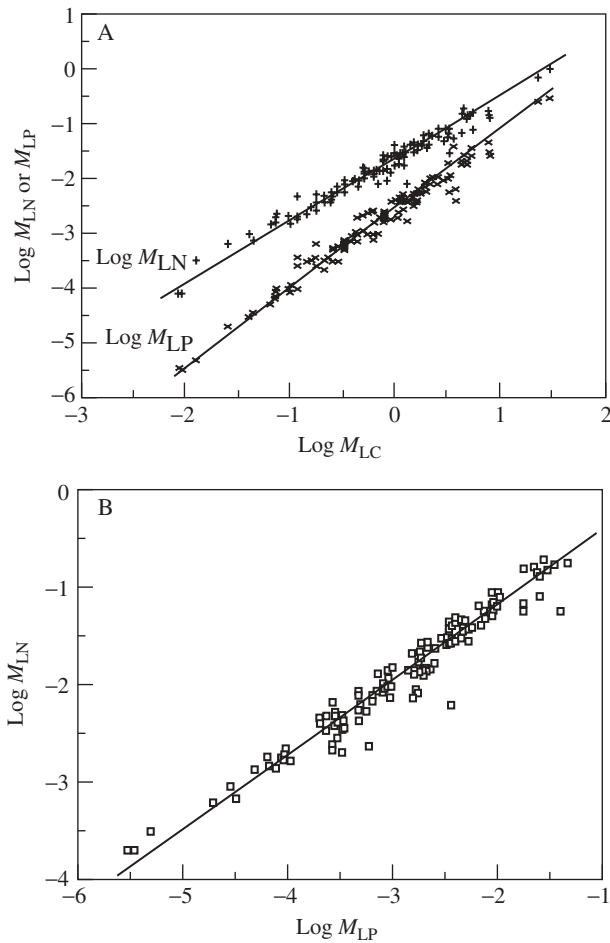
FIG. 3. Log–log bivariate plots illustrating the allometric relationships between total leaf nitrogen, phosphorus, and carbon content (M_{LN}, M_{LP} and M_{LC}, respectively) for 131 vascular plant species (for details, see Niklas *et al*., 2005). Lines are reduced major axis regression curves (for summary regression statistics, see Table 2).

FIG. 4. Log–log bivariate plots illustrating the allometric relationships between total leaf nitrogen, phosphorus and carbon contents (M_{LN}, M_{LP}, and M_{LC}, respectively). (A) Data for 17 specimens of *Eranthis hyemalis* (for details, see Niklas and Cobb, 2005). (B) Data for a world-wide collection of leaves (for details, see Niklas *et al*., 2005). Lines are reduced major axis regression curves (for a summary of regression statistics, see Table 2).

power of leaf carbon content (Fig. 3A and Table 2). For these species, it follows from $M_{LN} \propto M_{LC}$ and $M_{LP} \propto M_{LC}^{4/3}$ that $M_{LN} \propto M_{LP}^{3/4}$ (Fig. 3B and Table 2). Although stoichiometric analyses of plant conspecifics differing in size are sparse, those few data that are available indicate that intraspecific trends may abide by the same 'rules'. For example, in a recent study of *Eranthis hyemalis* (a perennial member of the Ranunculaceae), Niklas and Cobb (2005) report scaling exponents for M_{LN}, M_{LP} and M_{LC} relationships that are statistically indistinguishable from the proportional relationships $M_{LN} \propto M_{LC}$ and $M_{LP} \propto M_{LC}^{4/3}$ and $M_{LN} \propto M_{LP}^{3/4}$ (Fig. 4A and Table 2).

Whether these scaling relationships are 'universal' properties of vascular plant biology remains problematic. Based on an extensive world-wide survey of leaf N and P composition, Wright *et al*. (2004) report that leaf nitrogen scales roughly as the two-thirds power of leaf phosphorus content. In contrast, using an expanded version of the leaf N and P data reported by Reich and Oleksyn (2004) (consisting of 7445 entries for individual species reflecting conspecifics differing in age), regression analysis of M_{LN}
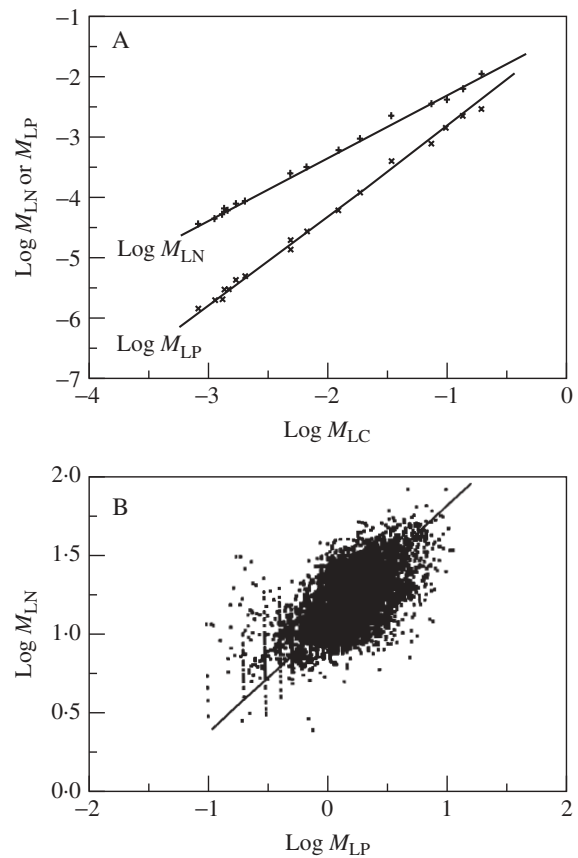
versus M_{LP} yields a scaling exponent of 0·73 with confidence intervals that contain the numerical value of three-quarters but exclude that of two-thirds (Fig. 4B and Table 2). This inconsistency may be the result of phyletic effects (i.e. biases introduced by differences in the taxonomic composition of the data sets used), but, regardless of the reason, it remains clear that the relationship between leaf N and P content is allometric and governed by the generic formula

$$M_{LN} = \beta_3 M_{LP}^{\alpha<1\cdot0} \qquad (10)$$

GROWTH AND A SIMPLE N, P-MODEL

Equation (10) takes on significance when it is wedded to a recently developed stoichiometric model for predicting the relative growth rates of diverse organisms based on their cell or tissue N and P contents.

Dobberfuhl (1999) first proposed that growth depends on total body nitrogen (N_T) and total body phosphorus (P_T) allocation patterns to protein and ribosomal RNA (rRNA) construction, respectively (also see Sterner and Elser, 2002; Ågren, 2004; Vrede *et al*., 2004). This model conceptually relates relative growth rates to N, P stoichiometry by

envisioning proteins as the 'overhead' that is required to achieve growth and rRNA as the protein-output 'machinery' required to maintain or recycle it. Dobberfuhl and others noted that, when an organism maintains a constant chemical composition, its relative growth rate μ can be mathematically expressed in terms of the amounts and rates of change of carbon C, nitrogen N and phosphorus P content by the formula

$$\mu = \frac{1}{C}\left(\frac{dC}{dt}\right) = \frac{1}{N}\left(\frac{dN}{dt}\right) = \frac{1}{P}\left(\frac{dP}{dt}\right) \qquad (11)$$

For any one of these essential substances, designated here by X, eqn (11) can be approximated by

$$\mu = \frac{1}{X}\left(\frac{dX}{dt}\right) = \ln\left(\frac{X_2}{X_1}\right) \times (t_2 - t_1)^{-1} \qquad (12)$$

where X_2 is the total cell, tissue or organismal concentration of substance x at time t_2 and X_1 is the total concentration of x at time t_1 (see Hunt, 1990). If the substance x is some measure of protein synthesis, eqn (12) can be recast as

$$\mu = \ln\left[\frac{f_N N_T + \left(\frac{k_s r_e F f_P P_T}{m_r}\right)}{f_N N_T}\right] \times t^{-1} \qquad (13)$$

where f_N is the decimal fraction of N_T invested in proteins, k_s is the protein synthesis rate per ribosome, r_e is the protein retention efficiency, F is the decimal fraction of total RNA allocated to rRNA, f_P is the decimal fraction of P_T invested in RNA, m_r is the mass of an average ribosome, and henceforth t denotes the time interval $t_2 - t_1$ (Dobberfuhl, 1999; Vrede *et al.*, 2004).

Using estimates or published numerical values of the variables required by this model, eqn (13) (or its variants) has been used to successfully predict the relative growth rates of different unicellular algae and small aquatic animals (e.g. Nielsen *et al.*, 1996; Klausmeier *et al.*, 2004; Vrede *et al.*, 2004) despite the assumptions that N_T and P_T allocation patterns are ontogenetically invariant, that balanced growth has been achieved, and the supposition that resources are not limiting.

Importantly, the complex stoichiometry redacted by eqn (13) can be integrated with allometric theory by noting that the ability to harvest light scales isometrically with respect to total annual plant growth across unicellular algae and vascular plant species (see Table 1; Niklas and Enquist, 2001). For vascular plants, this ability is gauged by standing leaf dry mass M_L. It is not unreasonable to suppose therefore that the relative growth rate of leaves μ_L may provide a reliable gauge of the relative growth rate of the entire plant body. Accordingly, if eqn (13) is *generally* valid across all manner of life forms, μ_L should be governed by total leaf nitrogen and phosphorus such that eqn (13) takes the form

$$\mu_L = \ln\left[\frac{f_N M_{LN} + \left(\frac{k_s r_e F f_P M_{LP}}{m_r}\right)}{f_N M_{LN}}\right] \times t^{-1}$$

$$= \ln\left[1 + \frac{k_s r_e F}{m_r}\left(\frac{f_P}{f_N}\right)\left(\frac{M_{LP}}{M_{LN}}\right)\right] \times t^{-1} \qquad (14)$$

Finally, combining this last formula with eqn (10) provides a quantitative description of leaf relative growth rates in terms of the allometry of total leaf nitrogen and phosphorus:

$$\mu_L = \ln\left[1 + \left(\frac{k_s r_e F}{m_r}\right)\left(\frac{f_P}{f_N}\right)\frac{M_{LP}^{1-\alpha}}{\beta_3}\right] \times t^{-1}$$

$$= \ln\left[1 + \left(\frac{k_s r_e F}{m_r}\right)\left(\frac{f_P}{f_N}\right)\frac{M_{LN}^{\frac{1}{\alpha}-1}}{\beta_3^{\frac{1}{\alpha}}}\right] \times t^{-1} \qquad (15)$$

Note that eqn (15) predicts that leaf relative growth rates will increase across species with either increasing leaf nitrogen or phosphorus allocations if and only if $\alpha < 1.0$.

TESTING THE MODEL

Equation (15) has three significant attributes. First, it directly incorporates an allometric relationship for leaf nitrogen and phosphorus allocation; secondly, it relates the N, P-stoichiometry of leaves directly to relative growth rates (and thus prior allometric theory treating the relationship between leaf dry mass and total plant annual growth); and, thirdly, it can be examined empirically based on observed leaf growth rates, thereby setting limits on the numerical values of α and other allometric or physiological parameters.

However, this model can be evaluated empirically only if the numerical values of all physiological variables are stipulated. For bacteria and animals, the values of some of these variables are comparatively well known, i.e. $k_s = 2.5 \times 10^{-11}$ µg protein ribosome^{-1} d^{-1}, $r_e = 0.60$, $F = 0.80$ and $m_r = 4.53 \times 10^{-12}$ µg rRNA ribosome^{-1} (Campana and Schwartz, 1981; McKee and Knowles, 1987; Mathers *et al.*, 1993; Sadava, 1993; Vrede *et al.*, 2004). Assuming that these values are equally applicable to plants, it follows that $k_s r_e F/m_r = 2.648$. Prior work also shows that between 16 % and 27 % of total leaf nitrogen is incorporated in Rubisco (Evans, 1989) and that, depending on whether ambient light conditions are high or low, between 15 % and 60 % of total leaf nitrogen is found in chloroplast thylakoids [pigment–protein complexes, electron transport constituents, reaction centres, components of the electron transport chain (particularly cyto *b/f*) and ferredoxin] (Evans, 1989). Based on published nitrogen allocation to Rubisco and thylakoids, it is reasonable to suppose that $f_N \sim 0.55$ across otherwise diverse species.

Finally, from prior analyses of 131 herbaceous species, the allometry of leaf nitrogen with respect to leaf phosphorus is reasonably well approximated by the formula $M_{LN} = 0.18 M_{LP}^{3/4}$ (see Table 2 and Fig. 3B) (Niklas *et al.*, 2005). Inserting these values into eqn (15) gives a model for the relative growth rates of leaves that lack the numerical value of only one parameter, the decimal fraction of total leaf P contributing to RNA:

$$\mu_L = \ln\left[1 + 47.37 f_p M_{LN}^{1/3}\right] \times t^{-1}$$

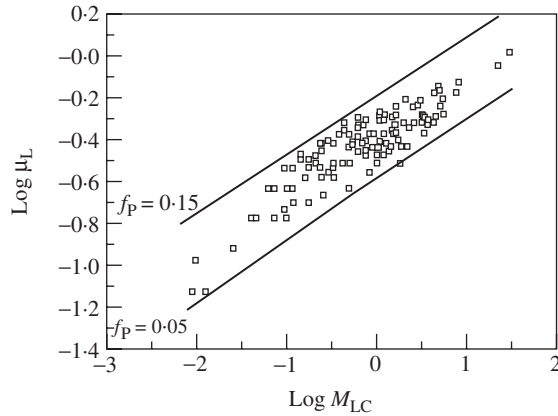$$= \ln\left[1 + 26.75 f_p M_{LP}^{1/4}\right] \times t^{-1} \qquad (16)$$

FIG. 5. Log–log bivariate plot of relative leaf growth rate versus total leaf carbon content (μ_L and M_{LC}, respectively) for 131 herbaceous species (for details, see Niklas *et al.*, 2005). The continuous lines denote predicted μ_L using eqn (16) and assuming that the decimal fraction of total leaf phosphorus allocated to the construction of RNA (f_P) equals 0·05 and 0·15.

FIG. 6. Log–log bivariate plot of relative leaf growth rate versus the quotient of total leaf nitrogen and phosphorus content (μ_L and M_{LN}/M_{LP}, respectively) for 131 herbaceous species (for details, see Niklas *et al.*, 2005).

Assuming that this decimal fraction varies little or not at all across species, this model predicts that μ_L will increase as a function of either increasing M_{LN} or M_{LP} that, in turn, should increase with leaf size as measured in terms of leaf carbon mass. It also predicts that μ_L should decrease as a function of increasing M_{LN}/M_{LP}. Unfortunately, reliable estimates for f_P for vascular plant species are currently unavailable. However, eqn (16) can be used to calculate μ_L using different values of f_P and the results can, in turn, be compared with observed leaf growth rates to set limits on the range of f_P-values that may occur in leaves.

Despite its simplicity and numerous assumptions, the behaviour of the model accords reasonably well with empirical trends observed for 131 ecologically and phyletically diverse herbaceous species (for details, see Niklas *et al.*, 2005). For these plants, observed μ_L scales as the 0·33 power of total leaf nitrogen ($r^2 = 0·72$), as the 0·25 power of total leaf phosphorus ($r^2 = 0·76$) and as the 0·22 power of total leaf carbon mass ($r^2 = 0·78$; see Fig. 5). Likewise, as predicted, μ_L decreases as the quotient M_{LN}/M_{LP} increases (Fig. 6). And, finally, the OLS regression curve for predicted versus observed μ_L is log–log linear with a slope very near unity.

CAVEATS

Nevertheless, considerable variation exists when observed leaf growth rates are plotted as a function of leaf carbon mass (see Fig. 3A). This feature can be attributed to a number of factors not addressed in the model but that are nevertheless of great ecological importance. Among the more obvious of these are species-specific differences or ecotypic variation in protein synthesis rates or retention efficiencies (Mathers *et al.*, 1993; Sadava, 1993; Güsewell, 2004), differences in the fractional allocation of leaf nitrogen to proteins as a consequence of leaf age or different ambient light intensities (Evans, 1989; Ryser *et al.*, 1997), morphological and anatomical differences in leaf
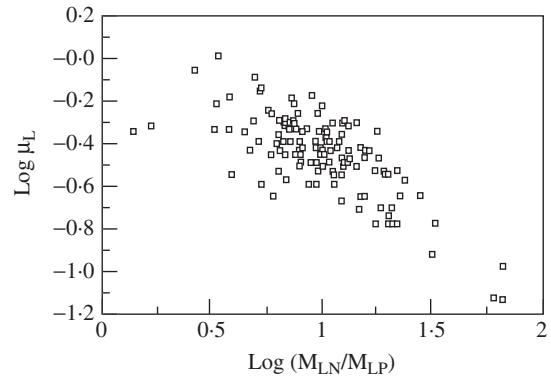
construction (Nielsen *et al.*, 1996), recruitment of N and P from older organs during early growth (Meyer and Tukey, 1965), differences in leaf tissue ploidy and nitrogen-use efficiency (Brown, 1978), and changes in N or P allocation to leaf components during leaf ontogeny.

Despite the remarkable success of the model in light of the undoubted influence of these and other physiological and ecological variables on growth, there are grounds for concern. As noted, one of the attributes of eqn (16) is that it can be challenged empirically by comparing the numerical values of physiological variables used to predict observed growth rates with those that are actually reported in the literature. One of these variables is the decimal fraction of total body phosphorus allocated to RNA construction, denoted by f_P. Inspection of Fig. 5 indicates that the relative growth rates observed for 131 plant species plot between those predicted by eqn (16) when f_P is *a priori* set equal to 0·05 and 0·15. The fact that all observed leaf growth rates plot within the 'corridor' defined by these two values suggests that between 5 % and 15 % of total leaf phosphorus is invested in the construction of RNA.

However, this range does not resonate well with f_P-values reported for other life forms such as bacteria, small aquatic heterotrophs, or unicellular algae. For these species, the decimal fraction of total cell or body phosphorus committed to RNA ranges between 0·20 and 0·90 (with an average value of 0·50 for animals) (see Rhee, 1978; Elser *et al.*, 2003). Therefore, the range $0·05 \leqslant f_P \leqslant 0·15$ identified by the model to 'contain' the leaf growth rates of vascular plants is unusually low.

This discrepancy may be the result of systematically underestimating plant growth rates because of their ability to store and annually recycle large pools of N and P. Specifically, the relative growth rates plotted in Fig. 5 are based on the difference in leaf N and P levels measured early and late in the growing season (see Niklas *et al.*, 2005). In retrospect, this procedure may have introduced a systematic bias because there is substantial evidence that vascular plants recruit N and P from older organs as new tissues and organs are produced during the early growth season (e.g. Mochizuki and Hanada, 1958; Meyer and Tukey, 1965;

Taylor, 1967; Taylor and May, 1967; Niklas and Cobb, 2005) and resorb substantial quantifies of N and P toward the end of the growing season, e.g. an average of 50 % of total P is reabsorbed before leaf senescence (50 % of which comes from nucleic acid hydrolysis; see Chapin and Kedrowski, 1983; Aerts, 1996). Therefore, N and P levels in newly formed leaves may be significantly higher than those reached once constant growth rates are achieved (thus violating a basic assumption of the model), and N and P levels may be on the decline even before visible signs of leaf senescence. If leaf relative growth rates are systematically underestimated for these (or any other) reasons, the upper and lower f_P-levels identified by the model to fit the data would be likewise underestimated.

VARIATION AND FUTURE DIRECTIONS

That significant differences in leaf N,P-stoichiometry exist even among vascular plants is strongly suggested by the allometric and stoichiometric trends reviewed here. As noted, the relationships between total leaf mass, annual growth rates, and total body mass differ between non-woody and woody plants (see Table 1). Across non-woody plants, total leaf mass scales isometrically with respect to both annual growth rate and total body mass (i.e. $M_L \propto G_T \propto M_T$), whereas, across woody plants, leaf mass scales as the three-quarters power to total body mass (i.e. $M_L \propto G_T \propto M_T^{3/4}$). Yet, based on the data presented here and elsewhere, total leaf mass (in units of carbon mass) scales isometrically with respect to total leaf nitrogen and allometrically albeit roughly as the three-quarters power of total leaf phosphorus (i.e. $M_{LC} \propto M_{LN} \propto M_{LP}^{3/4}$) (see Table 2). Assuming that these relationships are definitive, it follows that total annual growth rates should scale across all species as the three-quarters power of total leaf phosphorus across (i.e. $G_T \propto M_{LP}^{3/4}$). However, it also follows that total leaf phosphorus should increase as the four/thirds power of the total body mass for non-woody plants (i.e. $M_{LP} \propto M_T^{4/3}$) but scale isometrically with respect to the body mass for woody plants (i.e. $M_{LP} \propto M_T$).

These predictions have yet to be explored empirically, but they are in accord with the observation that much of the total body mass of woody plants is composed of physiologically inert material (heartwood) that increases in volume fraction with each passing year. Indeed, we need to know much more about the allometry and stoichiometry of what may be called 'necromass'—organic constituents that contribute to total body mass but that do not participate in metabolic activity or resource utilization, e.g. cell wall materials and secondary metabolites sequestered in the lumens of dead cells, which continue to accumulate throughout the lifetime of the multicellular individual. We also need to know much more about the allometry of annual biomass accumulation with respect to the N, P stoichiometry of meristematic tissues, both for herbaceous non-woody and woody species.

It is also clear that the juxtaposition of allometric theory and observation with the potential insights gained from N, P stoichiometric models is in its infancy. This approach clearly offers great promise (if for no other reason than that it helps to identify and quantify interdependencies across every level of biological organization, from molecules to ecosystems, and across bacteria to multicellular eukaryotes) but it is perhaps best viewed as a heuristic device with which to explore important conceptual issues. To be more effective, this juxtaposition would benefit greatly from more detailed measurements of how stoichiometric parameters vary ontogenetically and phylogenetically. In particular, more detailed data sets are needed for protein synthesis rates per ribosome, protein retention efficiencies, and the proportion of total P and N committed, respectively, to the construction of rRNA and non-structural proteins. Mutants of unicellular algae, like those of *Chlamydomonas*, and parasitic plants with a 'leaf-stem' construction, like *Monotropa*, should be used to 'dissect' how total N and P cell or tissue contents are allocated to the construction of different parts of the photosynthetic machinery. Perhaps in this way, we will be able to explain why tissue and organ nitrogen levels and dry mass scale as the three-quarters power of phosphorus and why so many other phenomena seem to obey similar quarter-power rules.

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